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In Vitro Antifungal Susceptibilities of Five Species of *Sporothrix*▽

Rita Marimon, Carolina Serena, Josepa Gené, Josep Cano, and Josep Guarro*

Unitat de Microbiologia, Facultat de Medicina i Ciéncies de la Salut, Universitat Rovira i Virgili, Reus, Spain

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Ninety-two isolates belonging to five species of the *Sporothrix schenckii* complex were tested in vitro against 12 antifungal agents, using a reference microdilution method. There were significant differences among the species; *Sporothrix brasiliensis* was the species that showed the best response to antifungals, and *S. mexicana* had the worst response. In general, terbinafine was the most active drug, followed by ketoconazole and posaconazole.

Sporotrichosis is a worldwide subacute or chronic infection caused by the dimorphic fungus *Sporothrix schenckii*, affecting both animals and humans. This disease is characterized by nodular cutaneous and subcutaneous lesions, which may involve the adjacent lymphatic system (2, 18). A saturated solution of potassium iodide has been used as an effective therapy for localized sporothrichosis. Other drugs commonly used are itraconazole (ITC) for the treatment of lymphocutaneous infections (1, 11, 20) and amphotericin B (AMB) for severe infections or when ITC therapy fails (9). Although these drugs are generally effective, the long duration of therapy and the toxicity of AMB make it necessary to explore new alternatives for the treatment of severe infections.

Some in vitro studies have demonstrated variable results among the strains tested, and some authors have concluded that antifungal susceptibility is strain dependent (7, 14, 21). This could be explained by the fact that *S. schenckii* does not represent a single species; instead it is a complex of cryptic species. Recently, using a multilocus sequence analysis, we have demonstrated that at least six phylogenetic species are included in the complex (13), several of these species being phenotypically characterized (12). Since the antifungal susceptibility of these species is unknown, we have evaluated the in vitro activity of 12 drugs against the mycelial phase of 92 strains representing five species of the complex (Table 1), using a reference microdilution method (15). The isolates were selected to represent the widest variety of geographical regions possible.

The isolates tested in this study were stored on potato dextrose agar (PDA) plates (Difco Laboratories, Detroit, MI) covered with paraffin oil, subcultured on PDA, and incubated at 30°C for 5 to 6 days. *Candida krusei* ATCC 6258 and *Candida parapsilosis* ATCC 22019 were used as control strains.

The antifungal agents were obtained as pure powders. AMB (USP; Rockville, MD), ITC and ketoconazole (KTC) (Janssen Pharmaceutica, Beerse, Belgium), albaconazole (ABC) (J. Uriach & Cía, Barcelona, Spain), voriconazole (VRC) (Pfizer, Inc., NY), posaconazole (PSC) (Schering-Plough, Kenilworth, NJ), ravuconazole (RVC) (Bristol-Myers Squibb Company, New Brunswick, NJ), eberconazole (EBC) (Laboratorios Salvat, S.A., Barcelona, Spain), and terbinafine (TRB) (Novartis, Basel, Switzerland) were diluted in dimethyl sulfoxide (Panreac Química S.A., Barcelona, Spain), and micafungin (MFG) (Astellas Pharma, Inc., Tokyo, Japan), flucytosine (5FC) (Sigma-Aldrich Corp., St. Louis, MO), and fluconazole (FLC) (Pfizer, Inc., Madrid, Spain) in sterile distilled water. Microplates were prepared as described in NCCLS standard M38-A (15). Final drug concentrations ranged from 128 to 0.25 μg/ml for MFG, from 64 to 0.12 μg/ml for FLC and 5FC, and from 16 to 0.03 μg/ml for the other drugs. The inoculum was prepared as recommended by the CLSI (formerly NCCLS) (15), by flooding the surface of the agar plate with sterile saline, scraping the sporulating mycelium with a culture loop, and drawing up the resultant suspension with a sterile Pasteur pipette. The suspensions were then filtered once through sterile gauze to remove hyphae. The numbers of conidia in the suspensions were adjusted to optical densities that ranged from 0.09 to 0.11, which corresponded to final concentrations of 1×10^4 to 5×10^4 CFU/ml. The viabilities of these inocula were verified by plating dilutions of the suspensions on PDA plates. The microplates were incubated at 30°C and read at 72 h. The MIC endpoint for the triazoles, AMB, MFG, and TRB was defined as the lowest concentration that produced complete inhibition of growth and for FLC, KTC, and 5FC as the lowest concentration that produced 50% growth inhibition. Approximately 80% of the tests were repeated and showed the same tendency (data not shown). However, in the few cases that did not coincide, the test was repeated and a modal MIC of the three values was considered.

The results are shown in Table 1. The MICs for the control strains agreed with the CLSI guidelines (15). TRB was the most active drug, showing a geometric mean (GM) MIC of 0.23 μg/ml for all the strains tested, followed by KTC with a GM MIC of 0.84 μg/ml. However, this latter drug was less active against *Sporothrix mexicana* (GM MIC of 4 μg/ml) and *Sporothrix albicans* (GM MIC of 3.2 μg/ml) than it was against the other species of the complex. The activity of KTC was more variable than that of TRB and depended on the species tested. PSC was the third-most-active antifungal drug tested, with a total GM MIC of 1.59 μg/ml, and the most active of the drugs for systemic use.

Although we could test only two isolates of *S. mexicana*, this

* Corresponding author. Mailing address: Unitat de Microbiologia, Facultat de Medicina i Ciències de la Salut, Universitat Rovira i Virgili, Carrer Sant Llorenç, 21.43201 Reus, Spain. Phone: 34-977-759359. Fax: 34-977-759322. E-mail: josep.guarro@urv.cat.

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TABLE 1. Antifungal activities of conventional and new antifungal drugs against 92 isolates belonging to species of the *S. schenckii* complex

Fungus (no. of isolates tested)	MIC[a] (μg/ml)	AMB	FLC	ITC	VRC	RVC	PSC	ABC	EBC	MFG	TRB	KTC	5FC
S. brasiliensis (23)	Range	1–4	128	0.5–2	0.5–16	0.06–2	0.25–1	0.25–4	0.06–1	256	0.06–0.25	0.06–0.5	2–128
	GM	1.67	128	0.7	3.88	0.44	0.62	1.13	0.26	256	0.09	0.15	14.49
	50%	2	128	0.5	4	0.5	0.5	1	0.25	256	0.06	0.125	16
	90%	4	128	1	8	1	1	4	1	256	0.25	0.25	128
S. schenckii (34)	Range	0.5–4	128	1–32	2–32	0.5–32	0.5–16	1–32	0.25–8	256	0.06–0.5	0.125–4	4–128
	GM	2.57	128	4	17.4	6.63	1.75	13.3	1.87	256	0.22	1	15.3
	50%	4	128	4	32	8	1	16	2	256	0.25	1	16
	90%	4	128	32	32	16	8	32	8	256	0.5	2	128
S. globosa (17)	Range	2–8	128	1–32	16–32	2–16	1–16	2–16	0.5–4	256	0.06–1	0.25–2	16–128
	GM	4.2	128	8.33	27.2	8	3.13	11.02	2.26	256	0.25	1.04	51.53
	50%	4	128	32	32	8	2	16	2	256	0.25	1	64
	90%	8	128	32	32	16	8	16	4	256	0.5	2	128
S. mexicana (2)	Range	16–32	128	32	16–32	32	32	32	32	256	0.5	4	128
	GM	20.16	128	32	32	32	32	32	32	256	0.5	4	128
S. albicans (16)	Range	2–8	128	32	4–32	8–32	1–2	8–32	2–16	256	0.25–4	2–8	128
	GM	5.18	128	32	11.8	19.03	1.91	18.38	9.51	256	0.8	3.2	128
	50%	4	128	32	8	16	2	16	8	256	0.5	4	128
	90%	8	128	32	32	32	2	32	16	256	4	4	128
Total (92)	Range	0.5–32	128	0.5–32	0.5–32	0.06–32	0.25–16	0.25–32	0.06–32	256	0.06–4	0.06–8	1–128
	GM	3.1	128	4.65	13.2	4.6	1.59	7.58	1.84	256	0.23	0.84	30.48
	50%	4	128	2	16	8	1	16	2	256	0.25	1	32
	90%	8	128	32	32	16	8	32	16	256	0.5	4	128

[a] GM, geometric mean; 50%, MIC at which 50% of the isolates were inhibited; 90%, MIC at which 90% of the isolates were inhibited.

was the species that responded least well to antifungals and only TRB showed a relatively low MIC (0.5 μg/ml) against this species.

FLC and MFG were not active against any of the isolates tested, as had already been demonstrated by other authors (11, 21). VRC showed poor activity, in agreement with the results of McGinnis et al. (14), who also obtained a high GM MIC (6.50 μg/ml) against strains of *S. schenckii*.

RVC and ITC only showed good activity against *Sporothrix brasiliensis*, whereas, for the other species tested, both drugs showed high MICs. Other authors (14) had also demonstrated poor in vitro activity of ITC. Despite these in vitro results, ITC has generally shown efficacy in the clinical setting. Conti Díaz et al. (1) successfully treated 18 cutaneous sporotrichosis patients with this drug. Sharkey-Mathis et al. (20) reported that 11 out of 15 (83%) patients with osteoarticular sporotrichosis who received ITC responded to the therapy.

In the present work, TRB has shown high activity against all the species tested. However, the therapeutic potential of TRB has been confirmed only for cutaneous and lymphocutaneous sporotrichosis (5, 6, 17, 19). By contrast, this drug has not demonstrated efficacy in the treatment of systemic sporotrichosis in a murine model (8). In severe or systemic infections, PSC constitutes a promising therapeutic agent since, in vitro, it has worked better than AMB and ITC, at least against strains of *S. brasiliensis*, *S. albicans*, and *S. schenckii*. Further in vivo studies are needed to confirm this activity.

Although in vitro results do not always correlate with in vivo outcome, the drugs tested showed in general very poor activity against *S. albicans*, *Sporothrix globosa*, and *S. mexicana*. It would be interesting to know if any drug combinations exert any activity against such species. However, no data are so far available on the activity of combined drugs against *S. schenckii* sensu lato.

In recent years, application of the phylogenetic species concept in different biological species of pathogenic molds has revealed different lineages that reflected species divergence (3, 10, 16). The delineation of these phylogenetic groups and the development of easy methods for their identification are crucial, since they can show different pathological behaviors and different antifungal responses (4). This study has demonstrated that *S. schenckii* constitutes a clear example of the latter.

Since clinical information on these new species does not yet exist, the significance of our findings is unknown. However, it seems that proper identification of the species of the *S. schenckii* complex involved in a given infection could be important for the appropriate treatment. For instance, in the case of a systemic infection, if the species causing the infection was *S. mexicana*, it is likely that the response to treatment with ITC or PSC would be poorer than if the species was *S. brasiliensis*.

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